



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US97/20444		[AT/US]; 3899 Haines Street #8-308, San Diego, CA 92109 (US). JURINKE, Christian [DE/DE]; Grope Hall 68, D-22115 Hamburg (DE). VAN DEN BOOM, Dirk [DE/DE]; Forsthausstrasse 8, D-63303 Preiech (DE). XIANG, Guobing [CN/US]; Apartment: 23, 11381 Zapata Avenue, San Diego, CA 92126 (US). LOUGH, David, M. [GB/GB]; 32 Deanhead Road, Eyemouth, Berwickshire TD14 55A (GB). (74) Agent: SEIDMAN, Stephanie, L.; Brown Martin Haller & McClain, 1660 Union Street, San Diego, CA 92101-2926 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
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(54) Title: DNA DIAGNOSTICS BASED ON MASS SPECTROMETRY			
(57) Abstract			
<p>Fast and highly accurate mass spectrometry-based processes for detecting a particular nucleic acid sequence in a biological sample are provided. Depending on the sequence to be detected, the processes can be used, for example, to diagnose a genetic disease or chromosomal abnormality; a predisposition to a disease or condition, infection by a pathogenic organism, or for determining identity or heredity.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/20444

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 C07H21/00 C07F9/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

29 July 1998

Date of mailing of the international search report

28.08.98

Name and mailing address of the ISA

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Osborne, H

INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 29431 A (SEQUENOM INC) 26 September 1996	1
X	pages 25-54	2-7,
	see page 9, line 33 - page 10, line 2;	11-18,
	claims 1-49; figure 9	82,83
X	see page 15, line 34 - page 18, line 10;	19-34,
	examples 5,8	82,83
X	see example 8	42
X	see page 16, line 4; figures 6A,8	47
X	page 36, ln 33	48,49,
	see page 26, line 7	80,81
X	see page 16 - page 18, line 10; figures 3-9	50-64,
		68-70
Y	see page 21, line 21 - line 23	35-37
Y	see example 7	38,39
Y	see claims 1-49	40,41,
		43-47,
		65-67,
		71-79

Y	WO 94 16101 A (KOESTER HUBERT) 21 July 1994	1-7,
		11-18,
		65-79,
		82,83
	see the whole document	

Y	WO 96 32504 A (UNIV BOSTON) 17 October 1996	1-7,
		11-18,
		66-79,
		82,83
	especially page 21, lns 12-24.	
	see the whole document	

Y	WO 95 13381 A (GERON CORP) 18 May 1995	35-37,
		82,83
	see page 9, line 1 - line 33	
	see page 29, line 34 - page 32, line 5	
	see claims 1-4; example 3	

Y	FENG J ET AL: "THE RNA COMPONENT OF HUMAN TELOMERASE" SCIENCE, vol. 269, no. 5228, 1 September 1995, pages 1236-1241, XP000645335	35-37,
	see page 1236, paragraph 1 - paragraph 4	82,83

Y	SYVANEN A -C ET AL: "DETECTION OF POINT MUTATIONS BY SOLID-PHASE METHODS" HUMAN MUTATION, vol. 3, no. 3, 1 January 1994, pages 172-179, XP000600258	35,38,
	see the whole document	39,82,83

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/20444

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PASINI B ET AL: "RET mutations in human disease" TRENDS IN GENETICS, vol. 12, no. 4, April 1996, pages 138-144, XP002072975 see page 141, paragraph 2 - page 144 ---	35,38, 39,82,83
Y	DE 44 31 174 A (DEUTSCHES KREBSFORSCH) 7 March 1996 see abstract and claim 1 ---	40,41
Y	NAITO H ET AL: "Detection of tyrosine hydroxylase mRNA and minimal Neuroblastoma cells by reverse transcription-polymerase chain reaction " EUROPEAN JOURNAL OF CANCER, vol. 27, June 1991, pages 762-65, XP002073102 see the whole document ---	40,41
Y	GB 2 260 811 A (YORKSHIRE CANCER RESEARCH CAMP ;UNIV LEEDS (GB)) 28 April 1993 see the whole document ---	40,41
Y	WO 96 17080 A (IMP CANCER RES TECH ;SELBY PETER JOHN (GB); BURCHILL SUSAN ANN (GB) 6 June 1996 see page 3, line 1 - line 2 ---	35,40, 41,82,83
Y	NELSON R ET AL: "Time-of-flight Mass spectrometry of nucleic acids by laser ablation and ionization from a frozen aqueous matrix " RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 4, September 1990, pages 348-351, XP002072976 see abstract ---	42,82,83
Y	TANG K ET AL: "MATRIX-ASSISTED LASER DESORPTION/IONIZATION OF RESTRICTION ENZYME-DIGESTED DNA" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 8, no. 2, February 1994, pages 183-186, XP000608266 see the whole document ---	42,82,83
Y	SIEGERT C ET AL: "Matrix-assisted laser desorption/ionization Time-of-flight Mass Spectrometry for the detection of polymerase chain reaction products containing 7-Deazapurine moieties" ANALYTICAL BIOCHEMISTRY, vol. 243, 1996, pages 55-65, XP002072977 see the whole document ---	42,82,83

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/20444

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 15400 A (UNIV JOHNS HOPKINS) 8 June 1995 see abstract and claims 1-19 ---	43-45, 82,83
Y	WO 96 10648 A (PROMEGA CORP) 11 April 1996 see claims 1,23 ---	43-45, 82,83
Y	WO 93 23563 A (CEMU BIOTEKNIK AB ;UHLEN MATHIAS (SE); PETTERSSON BERTIL (SE)) 25 November 1993 see claims 1-7; figure 1 ---	46,82,83
Y	DE 44 38 630 A (PACHMANN KATHARINA DR ;GOEHLY URSULA (DE)) 2 May 1996 see claim 1; figures 1A,1B ---	46,82,83
Y	EP 0 593 789 A (SUMITOMO METAL IND) 27 April 1994 see abstract, claims 1 and 2 ---	46,82,83
Y	WO 96 15262 A (MEDINNOVA SF ;DZIEGLEWSKA HANNA EVA (GB); BREIVIK JARLE (NO); GAUD) 23 May 1996 see page 12, paragraph 4 - page 13, paragraph 2 ---	46,82,83
Y	WO 89 06700 A (GENENTECH INC) 27 July 1989 see the whole document ---	47,82,83
Y	WO 89 03432 A (US ENERGY) 20 April 1989 see claims 1-21; figures 1,2 ---	65,68-70
Y	US 5 288 644 A (BEAVIS RONALD C ET AL) 22 February 1994 see claims 1-8 ---	66,67
X	WO 94 21822 A (KOESTER HUBERT) 29 September 1994 see claims 1-55 ---	65-79
X	US 5 430 136 A (URDEA MICHAEL S ET AL) 4 July 1995 see column 4, line 28 - column 9, line 52 ---	84-87
X	WO 95 31429 A (UNIV BOSTON) 23 November 1995 see claims 1,19 ---	84-87

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/20444

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ORDOUKHANIAN P ET AL: "Design and synthesis of a versatile photocleavable DNA building block. application to phototriggered hybridization" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 117, 1995, pages 9570-71, XP002072978 see the whole document ---	84-87
E	WO 97 42348 A (SEQUENOM INC) 13 November 1997 see claims 1-48 -----	1,7-10

INTERNATIONAL SEARCH REPORT

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

SEE ANNEXES

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

it should be further noted that "claims 82-83" as designated herein refer to two claims 82 and two claims 83 which were filed as follows, claims 82, 83 followed by a second claim 82 and a second claim 83)

1. Claims 1-18, partially 82-83:

A method for determining the sequence of a target nucleic acid involving the generation of base specifically terminated fragments.

2. Claims 19-34, partially 82-83:

A method for detecting a target nucleic acid present in a biological sample based on a nested polymerase chain amplification reaction.

3. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting telomerase), claims 36 and 37, and partially 82-83:

An assay for the detection of neoplasia/malignancies based on telomerase specific extension of a substrate primer and a subsequent amplification of the telomerase specific extension product by PCR.

4. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting mutation of a proto-oncogene), claims 38 and 39, and partially claims 82-83:

An assay for the detection of neoplasia involving mutation analysis of mutant or wild-type alleles by primer extension reaction by a Sanger type sequencing protocol.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. Claim 35 partially (in that it relates to the detection of neoplasia/malignancies by detecting expression of a tumour-specific gene in a specific tissue type), claims 40 and 41, and partially claims 82-83:

An amplification based assay for the expression of the tyrosine hydroxylase gene in bone marrow cells as indicative of a neuroblastoma.

6. Claim 42, partially claims 82-83:

A method for directly detecting double stranded nucleic acid using Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry.

7. Claims 43-46, partially claims 82-83:

A method for comparing DNA relatedness by amplification of microsatellite DNA repeat sequences.

8. Claim 46, partially claims 82-83:

A method for detecting mutations based on target amplification using a primer that introduces a unique endonuclease restriction site into amplified target and a combination of a Sanger sequencing protocol and endonuclease digestion.

9. Claim 47, partially claims 82-83:

A method for the amplification and detection of a nucleic acid based on the synthesis of RNA using a primer containing a RNA polymerase promoter sequence.

10. Claims 48, 49, 80 and 81, partially 82-83:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Primers per se for mass spectrometry comprising a mass modifying moiety.

11. Claims 50-64, partially 68-70, partially 73-79, partially claims 82-83:

Methods for detecting a target nucleic acid sequence involving hybridisation to a detector oligonucleotide.

12. Claims 65-67, partially 68-70, 71-72, partially 73-79, partially claims 82-83:

Methods for determining a nucleic acid sequence involving exonuclease digestion.

13. Claims 84-94:

Photolabile linkers per se for use in immobilisation of nucleic acids to solid supports.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/20444

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9629431	A	26-09-1996	US 5605798 A AU 5365196 A CA 2214359 A EP 0815261 A	25-02-1997 08-10-1996 26-09-1996 07-01-1998
WO 9416101	A	21-07-1994	AU 5992994 A CA 2153387 A EP 0679196 A JP 8509857 T US 5547835 A US 5605798 A US 5691141 A	15-08-1994 21-07-1994 02-11-1995 22-10-1996 20-08-1996 25-02-1997 25-11-1997
WO 9632504	A	17-10-1996	AU 5544696 A EP 0830460 A	30-10-1996 25-03-1998
WO 9513381	A	18-05-1995	US 5645986 A US 5629154 A AU 1178195 A AU 682082 B AU 1209095 A AU 1330795 A AU 6058298 A CA 2173872 A EP 0728207 A JP 9502102 T WO 9513382 A US 5648215 A US 5686306 A US 5639613 A US 5693474 A WO 9513383 A	08-07-1997 13-05-1997 29-05-1995 18-09-1997 29-05-1995 29-05-1995 04-06-1998 18-05-1995 28-08-1996 04-03-1997 18-05-1995 15-07-1997 11-11-1997 17-06-1997 02-12-1997 18-05-1995
DE 4431174	A	07-03-1996	NONE	
GB 2260811	A	28-04-1993	NONE	
WO 9617080	A	06-06-1996	NONE	
WO 9515400	A	08-06-1995	NONE	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter Application No

PCT/US 97/20444

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9610648	A	11-04-1996	AU 3998195	A	26-04-1996
			CA 2118048	A	31-03-1996
WO 9323563	A	25-11-1993	AU 4068293	A	13-12-1993
			CA 2135606	A	25-11-1993
			EP 0641391	A	08-03-1995
			JP 8500725	T	30-01-1996
DE 4438630	A	02-05-1996	NONE		
EP 0593789	A	27-04-1994	JP 5308999	A	22-11-1993
			WO 9323567	A	25-11-1993
WO 9615262	A	23-05-1996	AU 3851495	A	06-06-1996
			CA 2205017	A	23-05-1996
			EP 0791074	A	27-08-1997
WO 8906700	A	27-07-1989	AU 3058589	A	11-08-1989
			DE 68908054	T	10-03-1994
			DK 463089	A	20-09-1989
			EP 0359789	A	28-03-1990
			JP 2006724	A	10-01-1990
			JP 2503054	T	27-09-1990
			NO 300782	B	21-07-1997
WO 8903432	A	20-04-1989	US 4962037	A	09-10-1990
			CA 1314247	A	09-03-1993
			DE 3854743	D	11-01-1996
			DE 3854743	T	09-05-1996
			EP 0381693	A	16-08-1990
			JP 3502041	T	16-05-1991
US 5288644	A	22-02-1994	US 5453247	A	26-09-1995
WO 9421822	A	29-09-1994	AU 687801	B	05-03-1998
			AU 6411694	A	11-10-1994
			CA 2158642	A	29-09-1994
			EP 0689610	A	03-01-1996
			JP 8507926	T	27-08-1996
			US 5622824	A	22-04-1997

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/20444

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5430136 A	04-07-1995	US 5258506 A	02-11-1993
		US 5118605 A	02-06-1992
		US 4775619 A	04-10-1988
		CA 2088257 A	28-01-1992
		EP 0543889 A	02-06-1993
		JP 8311091 A	26-11-1996
		JP 9031090 A	04-02-1997
		JP 2552048 B	06-11-1996
		PL 170146 B	31-10-1996
		PT 98488 A,B	29-05-1992
		WO 9202528 A	20-02-1992
		US 5545730 A	13-08-1996
		US 5578717 A	26-11-1996
		US 5552538 A	03-09-1996
		US 5367066 A	22-11-1994
		AT 133714 T	15-02-1996
		DE 3854969 D	14-03-1996
		DE 3854969 T	30-05-1996
		EP 0360940 A	04-04-1990
		EP 0703296 A	27-03-1996
		ES 2083955 T	01-05-1996
		JP 2092300 A	03-04-1990
		JP 2676535 B	17-11-1997
		US 5380833 A	10-01-1995
-----	-----	-----	-----
WO 9531429 A	23-11-1995	US 5643722 A	01-07-1997
		AU 2635995 A	05-12-1995
		CA 2189848 A	23-11-1995
		EP 0763009 A	19-03-1997
		JP 10500409 T	13-01-1998
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WO 9742348 A	13-11-1997	AU 3003497 A	26-11-1997
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